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Evidence for Ion-Templation During Macrocyclooligomerization of Depsipeptides

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Abstract

The ion-mediated Mitsunobu MacroCycloOligomerization (M-MCO) reaction of hydroxy acid depsipeptides provides small collections of cyclic depsipeptides with good mass recovery. The approach can produce good yields of a single macrocycle, or provide rapid access to multiple oligomeric macrocycles in good overall yield. While Lewis acidic alkali metal salts are known to play a role in the outcome of MCO reactions, it is unclear whether their effect is due to an organizational (e.g. templating) mechanism. Isothermal titration calorimetry (ITC) was used to study macrocycle-metal ion binding interactions, and this report correlates these thermodynamic measurements to the (kinetically-determined) size-distributions of depsipeptides formed during a Mitsunobu-based macrocyclooligomerization (MCO). Key trends have been identified in quantitative metal ion-cyclic depsipeptide binding affinity (K_a), enthalpy of binding (ΔH), and stoichiometry of complexation across discrete series of macrocycles, and provide the first analytical platform to rationally select a metal-ion template for a targeted size-regime of cyclic oligomeric depsipeptides.

Graphical Abstract

Introduction

Structurally complex and chemically diverse cyclic peptide and depsipeptide natural products are attractive scaffolds in the development of sensors,¹ new materials,² and therapeutics.³ Although synthetic methods using coupling reagents have been developed to produce these molecules and their derivatives on commercial scale, the waste generated by their preparation has long been recognized as a necessary evil.⁴ Additionally, the synthesis

Supplementary Information. Procedures, complete experimental details, and analytical data for all new compounds are provided.

of macrocyclic peptides and depsipeptides typically suffers from the disadvantages associated with the preparation of their linear precursors. In order to overcome the tedium associated with a linear approach and solid-phase synthesis, an oligomerization/macrocyclization approach has been used.^{5,6,7,8,9,10} Although oligomerizations can greatly decrease step-count, they are often low-yielding and size-nonselective. Because cyclic peptides and depsipeptides tend to be ionophoric in nature,^{11,12,13} metal salt templates have been added to macrocyclization reactions based on the hypothesis that the salts can bind their linear precursors and cause a conformationally enhanced rate of cyclization.^{14,15,16} This hypothesis has been extended to cyclooligomerization reactions in order to favor different size-distributions of cyclic peptide^{17,18} (Figure 1) and depsipeptide¹⁹ products, but absent from the field is a broadly effective and rational approach, coupled to a rigorous analytical platform that might rationalize the product size-distributions. The ultimate goal would be development of a tool to predictably select a template for a particular size-regime.

The development of an expedient synthesis¹⁹ of collections of natural product-like cyclodepsipeptides, particularly one that includes a means to manipulate complex structural elements (i.e. substituents and configuration) could be a powerful platform to explore chemical diversity for a broad range of specific applications.^{10,20,21,22,23} We reported an initial solution that includes a hybridization of enantioselective synthesis, Umpolung Amide Synthesis (UmAS), and an (apparent) ion-templated macrocyclooligomerization (MCO). A Mitsunobu reaction was adopted as the key transform for both oligomerization and macrocyclization processes. This stereospecific reaction between a carboxylic acid nucleophile and alcohol electrophile benefits from an irreversible substitution, and is therefore inherently kinetically governed. A key feature of this discovery, however, was that ionic salts affect the overall production efficiency of the MCO, as well as the ring-size selectivity for arrays of macrocyclic products. We wondered, however, whether the nature of the salt could be the basis for predicting how the distribution of ring sizes will change, presumably as an ion templates the process. The notable amplification of various ring sizes highlighted in Figure 2A, led to the hypothesis that size selectivity could be correlated to the binding interactions of each macrocycle with its most suitable templating alkali metal ion (Figure 2B).

The relation between amplification and template-binding is commonly explored in thermodynamically controlled macrocyclooligomerization for the creation of dynamic combinatorial libraries (DCLs).^{24,25,26,27,28,29,30,31} In a thermodynamically-driven³² templated oligomerization, the product distribution depends on the relative stabilities of the product-template complexes. A template that selectively binds one product can shift the equilibrium toward that species in high yields. However, optimization of an irreversible (kinetic) templated oligomerization reaction is quite different because the product distribution is dependent on multiple rates, including the template's role in transition state stabilization leading to each product.^{33,34,35} A kinetic template could bind early intermediates (lower oligomers) to slow their cyclization, while binding later intermediates (higher oligomers) to favor a conformation that undergoes more rapid cyclization. The driving-force behind faster cyclization to a select product in a kinetic templating reaction is that the template almost invariably binds the product more strongly than the precursor-template complex, so the product is also favored thermodynamically.³⁶ Therefore, studying

the relative binding properties of a range of templates to the products of a reaction is a practical way to infer their templating behavior.^{36,37}

Several methods are commonly used to elucidate the behavior of a metal complex in solution, such as UV-vis spectroscopy, NMR spectroscopy, soft-ionization MS spectrometry, and CD spectroscopy. However, these analytical methods are not always applicable, especially in the analysis of complexes that are disorderly, fragile, insoluble, paramagnetic, and/or have multiple binding modes.³⁸ In contrast, isothermal titration calorimetry (ITC) is a very powerful tool for studying the thermodynamics of a binding event. The endothermic or exothermic heat is directly measured from the reaction, thus enabling elucidation of an association constant (K_a) and the enthalpy (ΔH) of the reaction. Additionally, the ion:macrocycle stoichiometry of complexation (N) is determined by the inflection point of the resulting titration curve. Determination of these three parameters may provide a better thermodynamic characterization under optimal macrocycle-metal binding conditions.^{26,39} Because product formation in the ion-templated MCO is irreversible, the ability of the different metal cations to facilitate the efficient synthesis of particular product size distributions indicates that the transition states of their templated linear precursors have geometries^{40,41,42} that closely resemble the products. Thus, we speculated that the thermodynamic macrocycle-metal binding data obtained from ITC experiments might be correlated to the kinetic phenomena in the ion-templated MCO.^{33,37,39}

Results & Discussion

Experiment Design

The ion-templated MCO by Mitsunobu carbon-oxygen bond formation to create the ester linkage(s) is carried out in benzene, but the ITC experiments described here were conducted in methanol. Attempts to prepare ITC runs in other organic solvents and solvent mixtures, such as benzene, dichloromethane/acetonitrile (3:7), or acetonitrile, led to the observation that the salts alone and/or salt-macrocycle complex were not sufficiently soluble. As a result, consistent ITC data could not be obtained. Methanol is starkly different in polarity to benzene (solvent for MCO), and it is known that solvent does affect binding affinity.^{38,43,44} However, several accounts have shown that although binding constants do vary with solvent, the trend of their relative binding is consistent.^{45,46,47,48} Our goal was to observe, if possible, *trends in binding interactions as a function of macrocycle and cation size* in an attempt to rationalize the apparent MCO ion-templating effect. The determination of binding constants in the reaction solvent (benzene) was therefore deemed less important.

Normalized Amplification Factor (AF_n) Analysis

In order to correlate trends in binding interactions with changes in macrocycle size distribution, we needed to establish a term to determine which template-induced changes are significant. Normalized amplification factor (AF_n) is a unitless value between -1 (complete disappearance of product) and 1 (maximum possible product) to determine significance of increase in yield of individual products in the presence of a template, relative to the control and others formed in the reaction.^{49,50,51} This term is generally used to analyze DCL product mixtures because it accounts for the large size-regime of possible products. To

compare amplification factors between different products, the concentrations of the products are normalized on the basis of the maximum concentration of available monomers. Because AF_n is based on available monomer concentration, the term can be applied to ion-templated MCO when the available monomer concentration is normalized to represent the isolated yield of macrocycles in the reaction.^{30,31} The AF_n values for previously reported collections of macrocycles in the presence of metal ion templates are shown in Figure 3. In order to account for experimental variance, template-induced changes are deemed significant when $|AF_n| > 0.09$.^{49,52,53}

Correlation of ITC Measurements with MCO Salt Effects

In the ion-templated MCO approach to verticilide, ring-size selectivity largely varied with monomer size, even though the same D,L-24- and 36-membered rings are formed from each monomer. To better understand this difference in behavior, salt-macrocycle binding interactions of the macrocycles formed using tetradepsipeptide **2** and didepsipeptide **1** were determined by ITC (Figure 4 & Figure 5). The data from ITC can be used to rationalize why different amounts of 24- and 36-membered rings are produced from tetradepsipeptide monomer **2** and didepsipeptide monomer **1** in the presence of the same ion templates. Under salt-free conditions, tetradepsipeptide **2** inherently produces cyclodimer **4** as the major product, along with 36-membered ring **6** and 60-membered ring **7** in minor amounts. However, in the presence of Na^+ , **4** is formed exclusively. As expected, **4** strongly and favorably binds Na^+ in a 1:1 stoichiometry. However, **6** and **7** also bind Na^+ , but in 2:1 ion:macrocycle ratios. The selectivity observed in the reaction despite the measured Na^+ binding ability of the other two potential products can be explained by a *positive templating effect*.^{37,33} Na^+ promotes the preorganization^{14,26} of two building blocks (**2**), driven by favorable binding to the linear precursor to **4** in a conformation that significantly accelerates an inherently favorable ring-closure. Additionally, a combination of the inherently slow oligomerization of **2** and preorganization of a two- Na^+ ion cyclization template, can explain why oligomerization/cyclization to **6** is much slower than to **4**. Similar logic can be applied to arrive at a reason why didepsipeptide **1** did not provide the same results with Na^+ : the rate of intramolecular (Mitsunobu) cyclization of **1** is much faster than that for **2**, so although cyclization to **4** is greater than without salt conditions, the concentrated reaction conditions and more reactive monomer (**1**) renders intermolecular coupling a more competitive process than in the former example. Additionally, 18-membered ring **3** and 30-membered ring **5** were found to exist in an equilibrium of 1:2 Na^+ to macrocycle aggregates and 2:1 Na^+ to macrocycle complexes, averaging 1.5 bound ions.^{54,55} The observation of a 1:2 binding pattern with **3** can imply that Na^+ promotes the preorganization of two linear-precursors, which then rapidly undergo cyclodimerization to the 36-membered ring (**6**) with the help of a second Na^+ ion. Because **5** is substantially larger than **3**, formation of a 1:2 metal-linear peptide complex is more likely to result in stable aggregates, as opposed to the precursor of a cyclodimerization reaction. Thus, the ITC binding pattern observed with **5**, which has the strongest 2- Na^+ binding constant, can imply that cyclization of the linear precursor is slowed by the formation of aggregates, but is then accelerated when the complex picks up an additional Na^+ ion.

Moving to a slightly larger cation, K^+ , with tetradepsipeptide **2**, the 36-membered ring is now significantly increased while the 24-membered ring is decreased. The 36-membered ring has the greatest enthalpically favorable 1:1 binding to K^+ , but is not produced selectively, unlike the observation with the 24-membered ring and Na^+ . This can be explained by the overall efficacy of K^+ as a cyclization template. An effective template must have the capacity to bind early intermediates in the oligomerization, but also prevent them from competitively cyclizing.^{33,37} In this case, the untemplated cyclodimerization reaction is very fast, and addition of K^+ slows it, but not enough to selectively favor formation of the cyclotrimer (**6**). However, addition of K^+ to the MCO with **1** produces the 36-membered ring in nearly double its original amount. In conjunction with the inherent fast intermolecular oligomerization of monomer **1**, and strong binding to K^+ , the rate of 36-membered ring cyclization can be selectively increased. ITC indicates that the other ring sizes either weakly bind ($K_a < 1 \times 10^4 \text{ M}^{-1}$),⁵¹ or do not bind K^+ at detectable levels, so their cyclization rates and respective yields are minimally affected.

The largest cation (Cs^+) with tetradepsipeptide **2** significantly amplified both of the larger ring sizes relative to the control, but did not favor them substantially as observed with **4** and Na^+ . ITC data shows that **6** and **7** have similar, but weak, binding affinities to Cs^+ (Figure 4). However, using Cs^+ with didepsipeptide monomer **1** does not result in significant amplification of macrocycles relative to the salt-free control, aside from a modest increase in 36-membered ring (**6**). In these two examples, the modest change in size distribution can largely be attributed to the lack of a driving force from formation of a strong Cs^+ -binding product.

Correlation of ITC Measurements With Salt Effects and D,D-Macrocycles

The use of enantioselective catalysis to prepare the depsipeptide monomer provided a synthesis platform to investigate a broad scope of precursors that might exhibit a more diverse range of behaviors in the MCO reaction. It was more tractable, therefore, to question whether the trends observed with monomers **1** and **2** might also apply to monomers with different stereochemistry (Figure 6). Unlike its L,L-analog (**1**), L,D-monomer (**8**) inherently favored direct cyclization to the 6-membered ring (**9**), in addition to producing small amounts of 18-membered (**10**) and 24-membered rings (**11**) in salt-free conditions. However, addition of Na^+ doubled the amounts of both **10** and **11**, while the 6-membered ring (**9**) was significantly decreased. Additionally, a small amount of a 30-membered ring (**12**), which was not observed in salt-free conditions, was formed. Macrocycles **10**, **11**, and **12** strongly bind Na^+ , while **9** does not at detectable levels, which can explain why the rate of direct cyclization to **9** was so greatly decreased. Additionally, the binding affinities of **10** and **11** to Na^+ are strong enough to drive preorganization of their linear precursors and subsequent cyclization at a rate much faster than intermolecular oligomerization and cyclization to **12**, despite its slightly stronger binding affinity to Na^+ . Additionally, **12** exhibits a positive enthalpy of binding, suggesting that the binding is due to an unfavorable conformational change^{26,56,57} as opposed to hydrogen bonding. Because binding interactions of cyclized products are known to reflect their respective linear precursors, it can be inferred that the transition state leading to **12** binds Na^+ in a conformation that does not promote cyclization (*negative templating effect*),³³ which can explain why the rate of cyclization to **12** is not

more competitive with **10** and **11**. Addition of K^+ to monomer **8** decreased production of the monocyclused product, but only modestly increased the 30-membered ring (**12**) relative to the control and the other macrocyclic products. ITC data shows that product **11** weakly binds K^+ and that products **9**, **10**, and **12** do not bind at detectable levels. In this example, the modest change in size distribution can be attributed to the lack of a driving force from formation of a strong K^+ -binding product. The most notable results of this series were observed when Cs^+ was used as an additive: the 30-membered ring (**12**) was isolated as the major product, when it was not originally observed in salt-free conditions. The 30-membered ring (**12**) is the strongest 1:1 Cs^+ -binder, followed by the 24-membered ring, while **9** and **10** do not have detectable binding. Here, Cs^+ effectively accelerates the rate of cyclization to the two Cs^+ -binding macrocycles, which resulted in their increased production.

Prediction of Salt Effects Using Structural and Thermodynamic Trends

On the basis of the trends observed in Figures 4–6, the distribution of macrocycles using salt additives as a variable suggests that inherent structure-based reactivity can be inferred from ITC-analysis of macrocycle-salt binding. To demonstrate this principle, a new monomer (**13**) was synthesized⁵⁸ and its MCO products from the un-templated reaction were analyzed by ITC *before* running the MCOs with salts (Figure 8). Without salt, dipeptide **13** provided 18-, 24-, and 36-membered rings. The overall yield of the untemplated MCO using **13** was quite low (47%), likely due to the steric effect of $A^{1,3}$ -strain (from the *p*-chloro mandelic acid residue) on the Mitsunobu reaction. The low initial efficiency provided a workable starting point to study the effects of salts, especially those that might induce more reactive conformations by templation. The thermodynamic interactions of **14**, **15**, and **16** with various salts were measured by ITC, and predictions for how the size distribution might change in the presence of salts were made based on the structural and thermodynamic trends (Figure 7) observed in previous examples. With sodium, the 24-membered ring (**15**) has an order of magnitude stronger 1:1 binding affinity than the other two macrocycles, and thus, it should be amplified the most. Macrocycles **14** and **16** also have strong binding affinities to Na^+ , but are not expected to be greatly increased. Preorganization to form **15** will likely be faster than cyclization to **14**, and **16** requires a double-ion template, which will form more slowly than the single-ion template required for **15**. In general, **14**, **15**, and **16** have fairly weak binding affinities to the larger metal cations (K^+ and Cs^+), compared to the previously described examples. Macrocycles **14** and **16** both have enthalpically favorable, weak 1:1 binding affinities for K^+ , and **15** has a strong, enthalpically disfavored 2:1 binding affinity. The thermodynamically disfavored double-ion binding of the 24-membered ring may result in a diminished rate of cyclization, and potentially promote the formation of a 30-membered ring, which is *not* formed in the untemplated oligomerization. Lastly, **15** and **16** weakly bind Cs^+ , while **14** does not, which is hypothesized to result in modest amplification of both of the former.

Comparison of Predicted Salt Effects with Experimental Outcomes

Next, these predictions were compared to the outcomes when **13** was subjected to MCO conditions using the various ion templates. As shown in Figure 8, the size-distributions of

macrocycles formed in the presence of salts are largely aligned with the predictions made from ITC measurements. Out of the three salt additives, Na^+ and Cs^+ provided size-distributions that most closely resembled the predictions. The addition of Na^+ substantially amplified the strongest-binding macrocycle (**15**), and modestly increased **16**, while Cs^+ modestly increased **15** and **16**, as predicted. The template effect of K^+ was more difficult to predict because a 30-membered ring was not isolated from the salt-free conditions; hence, its binding affinity to K^+ is unknown, and the binding affinities of all three isolated macrocycles were weak. The modest amplification of **15**, concomitant with little to no amplification of the other two ring sizes relative to the control may be due to K^+ causing the reaction to stall at the 24-membered linear precursor. The 24-membered ring (**15**) has an enthalpically unfavorable double-ion binding to K^+ , which could mean that its linear precursor's rate of inter- and intramolecular coupling are both slowed, resulting in the reaction to stall at this intermediate. Because ion binding is a reversible process, the amplification of **15** could be due to untemplated cyclization of some of its accumulated linear precursor.

Conclusion

In summary, we have implemented ITC as a tool to study macrocycle-template binding interactions, and show that these measurements can be correlated to size-distributions of depsipeptides formed during Mitsunobu-based MCOs. These studies have identified key trends in quantitative metal ion-cyclic depsipeptide binding interactions across discreet collections of macrocycles. Importantly, these behaviors can be elucidated across collections of macrocycles formed from monomers of different sizes, stereochemistry, and side-chains. In general, the ITC data indicates that there is a trend where macrocycles with a strong template binding affinity ($K_a > 1 \times 10^4$) will be formed in substantially greater amounts in the presence of that template. Two particularly notable instances of this phenomenon were observed with **15**, which was doubled in the presence of Na^+ ($K_a = 1.12 \times 10^5 \text{ M}^{-1}$), and **12** which was formed as the major product in the presence of Cs^+ ($K_a = 1.12 \times 10^4 \text{ M}^{-1}$), but formed not at all without a salt. The stoichiometry and enthalpy parameters from the ITC data, in combination with relative binding affinities, provide us with a full picture on how the ion template will affect the size distribution of products in a collection of macrocycles. In general, a 1:1 metal ion:macrocycle complex observed by ITC indicates a faster rate of formation than what would be observed with a 2:1 complex, simply because preorganization of a 1-ion cyclization complex is faster than one that requires 2 ions. 1:2 Complexes have been observed by ITC, and were found to correlate with inhibited of monocyclization of smaller rings, and increased formation of their corresponding cyclodimer in the presence of the salt additive. However, when observed with larger rings, 1:2 complexes correlate with overall slowed cyclization. The enthalpy parameter served as a good indicator for whether or not cyclization to a particular ring size would be favorable in the presence of a given template. This parameter is especially important as a predictive measure if multiple macrocycles in a series have strong binding to a particular ion. In general, negative binding enthalpy with strong ion-affinity resulted in an increased rate of cyclization, and positive binding enthalpy with strong ion-affinity resulted in a slowed rate of cyclization. The thermodynamic trends described here provide the first analytical platform to rationally select a template for a targeted size-regime of cyclodepsipeptides. Our current efforts are directed

at broadening the synthetic impact of this method by continuing to expand our scope of cyclodepsipeptides, while uncovering more structure-related trends with ITC. Future studies will explore this tool further, perhaps as a measure to correlate antibiotic activity of cyclodepsipeptides with ion-binding selectivity.⁵⁹

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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52. Variance within control MCO experiments and within templated experiments was insignificant, and fell within range of reported DCL literature examples, so $AF_n > 0.09$ can represent significant templated amplification. See SI for details.
53. For ITC experiments, salts were chosen for their solubility in MeOH, to ensure accurate concentrations. Prior work suggests cation-dependent product size-distribution (ref. 19). Thus, counter-anions are not included in the figures for simplicity. However, specific salts used in ITC or MCO experiments are detailed in the SI.
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Figure 1.
Templated cyclooligomerization approaches for peptide synthesis

Figure 2.
 (A) Mitsunobu-based MCO highlights and (B) strategy for measurement-based macrocycle synthesis

Figure 3.

Normalized amplification factors (AF_n) induced by ion templates. Significant changes ($|AF_n| > 0.09$) are shown in bold.

Figure 4. Correlating ITC data with the effect of salts on collections of macrocycles formed using 2^a

^aSignificantly increased yields are shown in bold. ^bITC binding affinities <1x10³ M⁻¹ are not detected

Figure 5. Correlating ITC data with the effect of salts on collections of macrocycles formed using **1^a**

^a Significantly increased yields are shown in bold. ^bITC binding affinities <1x10³ M⁻¹ are not detected

Figure 6. Correlating ITC data with the effect of salts on collections of macrocycles formed with 8^a

^a Significantly increased yields are shown in bold. ^bITC binding affinities <1x10³ M⁻¹ are not detected

Figure 7.
Applying structural and thermodynamic trends to templated macrocycle synthesis by MCO

Figure 8. Using ITC to predict changes in product size distribution from 13^a

^a Significantly increased yields are shown in bold. ^b ITC binding affinities <1×10³ M⁻¹ are not detected